THESIS

"THE EFFECT OF BILE ON THE ABSORPTION OF RADIOACTIVE IODINE, (I^{131}), IN DOGS"

M. H. W. Friedman

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SYNOPSIS

The concept of an enterohepatic circulation of radioactive thyroidal substances is presented. Previous experimental evidence justifying this concept is reviewed to indicate the basis for this investigation. A method of study is proposed of the uptake of radioiodine in plasma and in lymph in control dogs, and in dogs with diversion of normal bile flow.

The physiology of lymph and of bile are briefly discussed.

The experimental section is divided into three chapters. The first chapter contains three parts, describing the standardization of the surgical techniques involved in cannulations of the thoracic duct to collect lymph, of the jugular vein to obtain blood samples, and of the common bile duct to divert bile flow.

The second chapter describes the experimental procedures and is divided into four parts. Part A deals with the preparation and grouping of the experimental animals. In Part B, the administration of radioactive iodine (I^{131}), to the groups of dogs is indicated. Parts C and D of this chapter describe the collection of samples of plasma, lymph and bile, and the counting and calculation of radioactivity in these samples.

Chapter III presents the results. A brief explanation of the presentation pointing out significant findings accompanies the tables of results.

Two final chapters, IV and V, present a discussion of the

findings and the conclusions. Nearly identical concentrations of radioactivity were found in plasma and lymph in each group of dogs. The absorption of radioactivity in intestinal lymph is discussed. A significantly lower uptake of radioiodine was found in the plasma and lymph in dogs with diverted flow of bile than in the control animals. Interference in the enterohepatic circulation of radioiodine is believed responsible for this reduced uptake.

The significance of the enterohepatic circulation in man, and clinical application of the results of the present study are briefly discussed. It is suggested that clinical biliary obstruction or fistulae will produce an abnormal uptake of radioiodine given as a diagnostic tracer dose.

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" THE EFFECT OF BILE ON THE ABSORPTION OF RADIOACTIVE IODINE, (I^{131}), IN DOGS. "

A Dissertation
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in Partial Fulfilment of the Requirements for the Degree
of Master of Science

in the

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by

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Under the direction of

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INTRODUCTION

The role of the gastrointestinal tract, including the liver, in the metabolism of thyroidal substances, has been debated for years. Since the availability of radioisotopes of iodine in 1946, it has been possible to administer tracer doses of these materials, and to follow their absorption and excretion by various tissues with radiation counters.

In 1947, Gross and Leblond[20] injected radiothyroxine into rats, and observed that the liver and gastrointestinal tract concentrated large quantities of the radioactive material. Further studies indicated that the radioactive material was transferred from the liver to the gastrointestinal tract mainly by way of the bile.[9,10]

Albert and Keating,[1,2] in 1952, elucidated the concept of an enterohepatic circulation of radioactive thyroidal materials. The initial concentration of radioactive material in the liver and gastrointestinal tract was shown to be independent of the route of administration. In rats, after administration, an immediate distribution of radioactivity occurred between the liver and gastrointestinal tract and the remainder of the body. Following this, a rapid diffusion of radioactivity occurred into the gastrointestinal tract, chiefly by way of the bile, but also by direct secretion. Albert and Keating concluded that the liver and the enterohepatic circulation played an active, but not fully understood role in the absorption and metabolism of thyroidal materials. This contradicted the previously held belief[37] that the liver only detoxified and excreted excessive quantities of thyroidal materials, just as it fulfils a similar function for many other substances.

Other workers have postulated a mutual functional relationship between the liver and thyroid gland.[12,26] This relationship has been studied by experimental interference in the enterohepatic circulation. Such interference has been produced by interruption of the 'afferent limb' of this circulation (consisting of the portal vein and lymphatic drainage), obstruction of the 'efferent limb' of the circulation (the common bile duct), and by alteration of the normal function of the liver.

Another method of study of the liver-thyroid relationship has been by stimulation or depression of the thyroid gland. Klitgaard,[26] in 1953, administered labelled thyroxine to normal rats, and to hypothyroid rats whose thyroid function had been depressed by thyroidectomy or thiouracil treatment. He reported a significant decrease in the amount of radioactive material that appeared in the bile of the hypothyroid rats as compared to the normal animals.

In 1953, Roche[42] demonstrated that in thyroidectomized rats, subcutaneously administered thyroxine disappeared more slowly from the blood stream than did similarly administered triiodothyronine. Following ligation of the common bile duct, thyroxine elimination was further retarded.[43]

Briggs,[47] in 1951, demonstrated the biliary excretion of radioactive thyroxine in the dog and the rat. In 1953[9] he reported the presence of a conjugated product of radiothyroxine (identified later as the glycuronide), in the bile of a surviving rat liver preparation after addition of I^{131} labelled thyroxine to the perfusate.

The presence not only of conjugates of both thyroxine and triiodothyronine, but also of further derivatives of triiodothyronine in the

...

Other workers [illegible] between the liver and thyroid gland. [illegible]

[illegible]

In 1951, [illegible]

[illegible]

[illegible]

bile of rats was established by chromatographic and specific chemical staining methods by Roche,[44] in 1954.

A study of the intracellular localization of labelled thyroxine by Lee,[30] in 1954, indicated that the thyroid hormone is actively concentrated by the liver. The role played by liver cell mitochondria and by Coenzyme A in the enterohepatic accumulation of thyroid hormone was demonstrated by Ringler,[41] in 1954, and by Klemperer,[24] in 1955.

These findings have thus established the active involvement of the liver in the metabolism of thyroid hormones.

Evidence of the inhibiting action of the liver on the function of thyroid gland tissue implanted in the liver in amphibious larvae and in mammals was reviewed by Doetsch,[12] in 1953.

Telkka,[50] in 1954, observed that the production of obstructive jaundice in adult male rats by ligation of their common bile ducts, resulted in a histological depression of their thyroid glands.

The effect of ligation of the common bile duct on the radioiodine uptake by the thyroid gland in rats was studied by Kowalewski et al,[27] in 1955. In these animals, there was a marked increase in radioactivity of the liver and a significant decrease in the uptake of radioiodine by the thyroid gland. The retention of radioactivity in the liver was apparently due to the absence of excretion of radioactive bile. The decreased uptake of radioactivity by the thyroid gland was believed due to interference in the 'efferent limb' of the enterohepatic circulation produced by ligation of the common bile duct.

In 1955, Kowalewski et al[28] provoked interference in the 'afferent limb' of the enterohepatic circulation in dogs by the creation of portocaval anastomoses (Eck fistulas). Following the administration of radioactive iodine, a significantly decreased uptake of radioactivity by the thyroid gland, and decreased radioactivity of the bile were found in Eck fistula dogs than in normal control animals. These results were considered due to an altered enterohepatic circulation of radioiodine. It could not, however, be determined if this altered circulation was due to a diminished transport of labelled material to the liver in the absence of a normal portal system, or to an impaired liver function with diminished ability of the liver to secrete radioiodine in the bile.

In a further study by Kowalewski et al,[29] in 1956, liver necrosis was produced in mice by administration of a hepatoxic agent (carbon tetrachloride). Following administration of radioiodine, an increase in radioactivity of the liver, and a decrease in the uptake of radioactivity by the thyroid gland was observed in each of these mice as compared to normal control animals. The results were considered due to interference in the enterohepatic circulation produced by the acute hepatic injury.

The role of the lymphatic system in this enterohepatic circulation has not been fully defined. Albert and Keating,[1,2] in 1952, presumed that the reabsorption and recirculation of radioactivity occurred from the bowel by way of the portal vein and lymphatic drainage.

The initial phase of the present investigation was undertaken to determine the uptake of radioactivity in lymph from the gastrointestinal tract, and in peripheral blood plasma during an acute study. Lymph was

proposed to be obtained from the gastrointestinal tract by cannulation of the thoracic duct in the neck of dogs.

Lymph has been defined[49] as a straw colored fluid consisting of a clear liquor with white blood cells which circulates in lymph spaces or lymphatic vessels. Lymph arising from intestinal lacteals is termed chyle. The fat content of chyle varies during the digestive process.

The route of lymphatic drainage from the intestine[22] passes from the central lacteal of each villus through a plexus of vessels piercing the bowel wall, to the larger lacteals of the mesentery that drain into the cysterna chyli. The thoracic duct arises from the cysterna chyli and empties into the left jugular vein at its junction with the left subclavian vein in the neck.

The physiology of the lymphatic system has been extensively described by Drinker.[14] He states: "Lymph is derived from the water and the dissolved substances that leave the blood capillaries, and to some extent from metabolites diffusing out of the tissues."

The chemical composition of lymph has been described by Mitchell[36]: "The chemical composition of lymph is qualitatively similar to that of plasma, but differs quantitatively. The concentration of proteins is lower, while the concentration of certain waste products and other diffusable metabolites tends to be higher in lymph than in plasma. This is due in part to the origin of lymph. It is to a considerable extent an exudate from the blood. Proteins are the most abundant solids of lymph, as they are of plasma, but only about half as concentrated. Those entering into the clotting process are not sufficiently concentrated to produce as firm a clot in lymph as in blood."

The cellular content of lymph is variable, but it is minimal in lymph such as chyle having a high fat content. The predominant cell present is the lymphocyte, but there are always some erythrocytes present. The number of erythrocytes in thoracic duct lymph can readily be increased by abdominal massage, and by passive and active movements of the extremities.[14]

The rate of lymph flow is variable,[14] and dependent on numerous factors, including activity of the part, blood pressure, hydration, oxygen and carbon dioxide levels, and physical factors such as heat and cold. Anesthesia has a depressant effect on the rate of lymph flow, but Drinker[14] states that under stable conditions using nembutal anesthesia in dogs, a steady flow of lymph may be obtained for several hours from cannulated lymphatics. He reports a mean lymph drainage of 4.3 millilitres per 10 minutes from the thoracic duct in dogs averaging 19 kilograms in weight, and under barbiturate anesthesia.

The second phase of the present study was undertaken to determine the effect of diversion of normal bile flow on the uptake of radioactivity in lymph from the gastrointestinal tract and in blood plasma, utilizing the initial series of animals as controls. Diversion of bile flow was proposed by cannulation of the common bile duct. The biliary system was decompressed by either removal of the gall bladder, or by drainage of the gall bladder with ligation of the cystic duct.

Bile has been defined[49] as the yellowish brown or green fluid secreted by the liver, stored in the gall bladder, and discharged into the duodenum.

The route of bile drainage[32] is from the liver cells through

intercellular canaliculi, to interlobular bile ducts. These intrahepatic ducts form an anastomotic network that drains into the right and left hepatic ducts. These ducts fuse externally to form the common hepatic duct. At the junction of the cystic duct from the gall bladder, the common hepatic duct becomes the common bile duct which empties into the duodenum.

Bell[5] lists the constituents of bile as water, solids, bile salts, bile pigments, cholesterol and inorganic salts. Gall bladder bile is more concentrated than bile from the hepatic ducts, and Best[8] states that in the gall bladder, water is absorbed and materials such as cholesterol and mucus are added.

Although bile is continuously secreted, the rate of flow into the intestine is intermittent.[8] Factors influencing the rate of flow include stimulants causing gall bladder contraction and substances directly increasing bile secretion. The most important of the latter are the bile salts, which normally undergo an enterohepatic circulation.[5] They pass in the bile into the duodenum, are largely reabsorbed from the bowel, and recirculated by way of the portal circulation, to be concentrated in the liver for resecretion into the bile. During withdrawal of bile through a fistula with consequent loss of bile salts, bile secretion gradually diminishes, but may be increased by introduction of bile salts into the intestine.

The dog has not been a popular experimental animal for radioiodine uptake studies. One disadvantage has been the belief, among some workers,[11,18] that the dog is less dependant on its thyroid gland for normal metabolic activities than other species. This belief arose due to the difficulty in producing evidence of hypothyroidism in dogs by the usual techniques of

surgical thyroidectomy or treatment with antithyroid drugs,[18,33] and also because plasma protein-bound iodine levels are low in this species.[11]

Goldberg,[19] however, in 1952, utilizing large doses of radioiodine, produced total radiothyroidectomy in several dogs. He demonstrated the later development of clinical myxedema, and of behavioural and pathological changes characteristic of hypothyroidism in these animals. Goldberg conceded that the dog may require only minute amounts of thyroid hormone for the maintenance of an apparently normal metabolic state, but maintained that hypothyroidism could be produced following total thyroidectomy in dogs.

The thyroid uptake of radioactive iodine in dogs was investigated by Fredrickson,[17] in 1955. He injected a tracer dose (5 - 10 microcuries), of radioiodine intravenously, and utilizing a specially constructed scintillation counter, he determined thyroid uptake by external counting in the unanesthetized animal. Maximum thyroid uptake was attained slowly in his series, reaching a peak at the third day. However, the reported average percentage of the tracer dose eventually detected in the thyroid gland of the dog was essentially the same as that in the human.

The dog was, therefore, considered an adequate experimental animal for use in the present investigation.

The final phase of the present study was undertaken to determine the effects on the uptake of radioactivity in plasma produced by the anesthetic and operative procedures of the initial phases. A series of control animals under physiological conditions was proposed for this portion of the study.

CHAPTER I

STANDARDIZATION OF SURGICAL TECHNIQUES

PART A

Cannulation of the Thoracic Duct

The operative technique for cannulation of the thoracic duct is described by Markowitz[31] as follows:

"This is a good exercise in careful dissection. It requires more skill than cannulating a small vein or artery, since the duct walls are very thin, and collapse when incised, owing to the escape of contained lymph. This exercise is facilitated if the animal is given about 200 mls. of vegetable oil and 200 mls. of milk by stomach tube about three hours previously. The addition of 30 grams of butter saturated with Sudan III makes the identification of the duct easier (Dragstedt and Dragstedt). The experiment is likely to fail if the animal has severe distemper. Such an animal is so dehydrated that the lymph flow is almost nil, with the result that it takes experience to identify the thoracic duct at the root of the neck.

"Under ether anesthesia, and with facilities for artificial respiration immediately available in case the pleura is punctured in the dissection, an incision is made slightly to one side of the left jugular vein, which is usually visible in a dog with a shaved neck. The vein is dissected down to its junction with the left subclavian, small tributaries being cut between ligatures. The point where the diminutive internal jugular vein empties into the common jugular is identified. At this point the cellular space over the apex of the left lung is opened up with the handle of a scalpel, and emptying into the venous cistern can usually be seen a milky white cord.

This should actually be traced to its entry into the venous cistern, as otherwise an enlarged cervical lymph trunk may be mistakenly cannulated.

"As soon as the duct is tied, it becomes distended. It is now cannulated in the typical manner. A Harvard arterial cannula of moderate size is inserted. This is connected with a paraffined tube of rubber that projects through the skin incision, which is closed in the usual manner. If clotting occurs in the tubing, the clot should be expressed from the tubing by manipulation with the fingers. If this is unsuccessful, the rubber tube should be disconnected from the cannula and flushed through. Occasionally the cannula itself becomes clotted, and must be cleaned. This can usually be done without removing it from the vessel. The surgeon should familiarize himself with the properties of lymph. In surgical literature, the term 'lymph' is applied to all forms of tissue fluid with the exception of blood."

Numerous trial dogs were operated upon in order to develop a reliable technique of cannulation, and it was found necessary to modify the procedure outlined by Markowitz in several respects.

Each animal received no food or water for at least 12 hours prior to anesthesia. This assured an essentially empty stomach for the experimental procedure, and facilitated pre-operative milk ingestion.

To aid in identification and cannulation of the thoracic duct, it was found necessary to administer whole milk to each animal orally, about one hour before operation. An arbitrary dose of 25 mls. of milk per kilogram body weight was administered to stimulate lymph flow so that the thoracic duct could be more readily identified in the neck dissection. The

additional pre-operative ingestion of butter, vegetable oils, or fat dyes,[13] was not found to enhance thoracic duct identification, nor was such a heavy fat meal well tolerated by many of the trial dogs.

The anesthetic agent used was powdered nembutal, converted prior to use into a 50 milligram per ml. solution in distilled water, and injected intravenously in a dose of 35 mls. per kilogram body weight. This was found to give a smooth, rapid induction, and surgical anesthesia for 2 to 4 hours. Anesthesia could readily be maintained beyond this period by additional intravenous injections of 1 to 2 mls. of the nembutal solution at hourly intervals or whenever necessary. Endotracheal intubation was routinely performed.

Each animal was restrained in a supine position with its neck extended and the head turned to the right, exposing the left side of the neck. The skin of the neck was incised obliquely from the sternum to the lateral border of the left sternomastoid muscle, crossing the path of the left external jugular vein. The incision was carried down to the jugular vein, which was freed to the junction of the left subclavian vein. Hemostasis was meticulous. The thoracic duct was usually identified at its termination in a widened ampulla that entered into the undersurface of the junction of left subclavian and jugular veins (Fig. 1). Several smaller lymphatics of the neck were often found to unite at this ampulla. These were ligated and divided. The thoracic duct was then ligated at the ampulla. Following this manoever, the duct became distended, and it was carefully freed distally for about one cm. distance. With care, entry into the pleural cavity could always be avoided.

Cannulation of the duct was found, after many attempts, to be best accomplished in the following manner: exerting slight tension on the duct by means of the tie on the ampulla, a blunt, thin-walled No. 17 or No. 18 hypodermic needle was gently injected into the duct. Through the lumen of this needle, a fine polythene tube (No. 50 or No. 60), was then threaded into the duct for a distance of 2 cms. to 5 cms. and tied into place. Fine serrations made previously on the polythene tube exterior provided a grip for the ligature that prevented the tube from slipping out of the duct. The tube was then anchored to adjacent tissue and brought out through the incision, or through a stab wound in a manner to prevent it from becoming kinked.

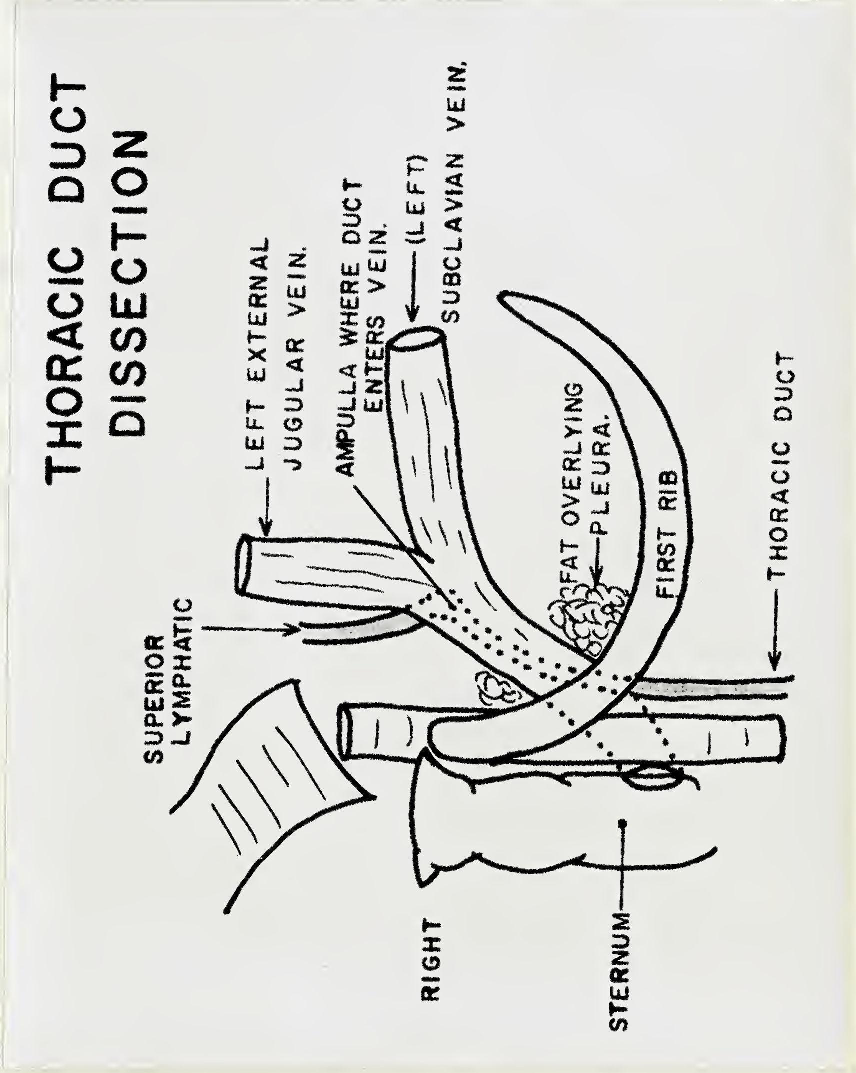
Lymph was allowed to drip freely from the end of the polythene cannula. The operative time for this procedure, following experience with the technique, averaged about 1.5 hours.

The clotting of lymph has been well described by Drinker.[14] Clotting tended to occur readily in the terminal thoracic duct or in the fine polythene tubes in operated animals following any obstruction to lymph flow. Attempts to collect quantitatively continuous lymph drainage from the thoracic duct in trial animals who were allowed to recover from anesthesia were unsatisfactory. General activity of the animals tended to kink and occlude the polythene cannulae, made frequent sampling and accurate lymph collection difficult, and introduced a risk of contamination of the samples by the animals' secretions following radioiodine administration.

These complications were avoided by maintaining the animals under nembutal anesthesia for the duration of the acute experiment. Clotting

was prevented by collection of lymph in centrifuge tubes heparinized prior to use with 1:1000 aqueous heparin solution allowed to dry as a film on the inner tube surface. The polythene tubing used for cannulation was also rinsed before use with this heparin solution.

FIGURE I


THORACIC DUCT DISSECTION
LEFT EXTERNAL JUGULAR VEIN.
AMPULLA WHERE DUCT ENTERS VEIN.
(LEFT) SUBCLAVIAN VEIN.
FAT OVERLYING PLEURA.
FIRST RIB
THORACIC DUCT
SUPERIOR LYMPHATIC
RIGHT
STERNUM

PART B

Cannulation of the Jugular Vein

To obtain samples of peripheral venous blood for determinations of radioactivity, the external jugular vein on the right side of the dog's neck was cannulated.

Under anesthesia, with the animal in a supine position, the neck was extended. Digital pressure applied suprasternally caused the vein to become distended. A large No. 13 hypodermic needle was stabbed through the skin and into the vein. As the needle entered the lumen of the vein, a fine No. 160 polythene tube was threaded through the needle barrel and centrally into the vein for several centimeters distance. This tube was filled with heparin solution diluted to 1:50,000 with normal saline to prevent clotting. The external end of the tube was then clamped. By releasing the clamp and removing the heparin solution, samples of blood were withdrawn at the required intervals into heparinized vacuum tubes.

PART C

Cannulation of the Common Bile Duct

Several operative techniques for draining bile externally are described by Markowitz.[31] The simplest technique is that of Rous and McMaster[45] in which the common bile duct is cannulated, and the bile drained externally, where it is collected in a sterile rubber balloon.

This technique is described as follows:

". . . . The tubing must be pliable to prevent its occlusion by kinking. It must be resistant as it passes through the abdominal wall to maintain its patency. In practice one uses a large, pointed glass cannula with a small flange at one end. This leads to pure gum tubing....... The gum tubing in turn leads to a U-shaped glass tube which is so adjusted that the bend lies near the brim of the pelvis. The other limb of the U tube is connected to firm stethoscope tubing, of which the walls are sufficiently rigid to prevent collapse by the pressure of the abdominal muscles. The free end of this tube leads to a toy balloon, which has a capacity of at least 200 mls. when deflated. A 'T' tube is inserted between the balloon and abdominal wall, and collections of bile are made by aspirating the balloon through this T tube.

". . . . A right rectus incision is made in the abdominal wall of a dog. The gall bladder area is exposed, and the common bile duct tied as close to the duodenum as possible. This is to prevent the remote possibility of regurgitation of infected duodenal contents. The gall bladder neck is severed between ligatures, care being taken not to interfere with the cystic artery. It is advisable to aspirate the bile in the gall bladder with a hypodermic needle and any oozing from the point of puncture is stopped with

a purse string suture. The cannula is now inserted in the usual manner into the common bile duct. The ligature for tying the cannula consists of medium silk twist, which has less tendency to cut through. The common duct is now severed below the cannula....... The abdominal incision is closed in the usual manner. "

It was found inadvisable in the present study to employ a permanent drainage of bile as described above. The technique was therefore modified in several respects.

Under nembutal anesthesia, the abdomen was entered through a midline incision. The liver was retracted, and the common bile duct identified at its entrance into the duodenum, usually 2 to 5 cms. distal to the pylorus. The common duct was then isolated and the entrance of the cystic duct identified. The cystic duct was doubly ligated.

In several animals the gall bladder presented readily. Following ligation of the cystic duct in these animals, the cystic artery was ligated and divided, and the gall bladder removed. In other dogs, the gall bladder was deeply embedded in the liver lobes, and removal was considered to be an excessively traumatic procedure. In these animals, utilizing a syringe and No. 21 hypodermic needle, the gall bladder was drained of its bile.

The common bile duct was ligated at its point of entrance into the duodenum. Proximal to this ligation, a longitudinal incision was made in the duct, and a fine No. 160 or No. 190 polythene tube was introduced into the duct so that the end of the tube lay distal to the junction of the hepatic ducts. The tube was tied in place with double circumferential silk sutures around the duct. The common duct was then divided proximal to its

entrance into the duodenum.

The free end of the polythene cannula was brought out through the incision. Bile was allowed to drain freely from the end of the tube. The abdomen was closed. Operative time for this procedure, following familiarity with the technique, averaged about one hour.

CHAPTER II

EXPERIMENTAL PROCEDURES

PART A

Preparation of Animals

Eighteen adult mongrel dogs of both sexes, weighing from 9 to 20 kilograms, were used in this investigation.

In the initial phase of the study, a series of 6 dogs was used to determine the uptake of radioiodine in lymph and in blood plasma. These animals comprised Group I, and consisted of dogs No. 1 to 6 (Table I).

In these animals, lymph was obtained by cannulation of the thoracic duct in the neck following the technique previously described. The jugular vein on the opposite side of the neck was cannulated for the purpose of blood sampling, using the technique described in Chapter I. The abdomen was opened through a midline incision, and a tracer dose of radioiodine was injected directly into the stomach. The abdomen was closed, and samples of lymph and plasma were collected for 12 hours following injection of radioiodine. The animals were maintained under anesthesia during this period. These dogs also served as controls for the following series described below.

In the second phase of this investigation, a series of 6 dogs was used to determine the effects of diversion of bile flow on the uptake of radioiodine in lymph and in blood plasma. These animals comprised Group II, and consisted of dogs No. 7 to 12 (Table I).

TABLE I

NUMBER, WEIGHT IN KILOGRAMS, AND SEX OF EACH ANIMAL IN 4 EXPERIMENTAL GROUPS OF DOGS					
GROUP I (6 Control Dogs)			GROUP II (6 Common Duct Dogs)		
NO.	WT.	SEX	NO.	WT.	SEX
1	15.5	MALE	7	14.0	FEMALE
2	10.0	MALE	8	15.0	MALE
3	11.0	MALE	9	19.0	MALE
4	11.5	MALE	10	20.0	MALE
5	10.5	FEMALE	11	19.5	MALE
6	20.0	FEMALE	12	20.0	MALE
GROUP III (3 Control Dogs)			GROUP IV (3 Control Dogs)		
NO.	WT.	SEX	NO.	WT.	SEX
13	9.0	FEMALE	16	12.5	MALE
14	18.5	MALE	17	15.5	FEMALE
15	15.5	FEMALE	18	17.0	MALE

In these dogs, lymph was similarly obtained by cannulation of the thoracic duct in the neck, and the jugular vein was similarly cannulated for blood sampling. In addition, however, the common bile duct was cannulated to divert the flow of bile, using the technique described in Chapter I. Following this procedure, radioiodine was similarly injected directly into the stomach, and the abdomen was closed. Samples of lymph and plasma were collected in all of these animals during 12 hours following injection of radioiodine. The animals were maintained under anesthesia during this period. Samples of bile were successfully collected in 3 animals (dogs No. 10, 11, 12) during the 12 hour period.

In the final phase of this investigation, a series of 6 dogs was used to determine any effects of radioiodine absorption by the milk ingestion prior to operation, operative trauma, prolonged anesthesia, and the moderate fluid loss caused by frequent sampling, in the previous two groups of dogs.

Three animals of this final series, dogs No. 13, 14 and 15, comprised Group III (Table I). These dogs received no milk pre-operatively. They were anesthetized with nembutal, and the jugular veins cannulated for blood sampling. Through an abdominal incision, radioiodine was injected directly into the stomach. These animals received no further anesthetic, and samples of blood were taken at infrequent intervals during 12 hours following radioiodine injection.

Dogs No. 16, 17 and 18 comprised Group IV (Table I). These animals were not anesthetized nor operated upon, and constituted a group of physiological controls. Radioiodine was given orally in their drinking

water, and samples of blood were taken from the peripheral veins at infrequent intervals during 12 hours following administration of radioiodine.

PART B

Administration of Radioactive Iodine

Daily iodine intake in the diet is important in any radioiodine study.[17] The animals in this investigation were maintained on commercial Miracle Dog Food prior to their use. The percentage of iodine in this dog food is known by analysis (Appendix I), and the daily dietary intake of iodine was found by calculation of an average ration to be about 1,000 micrograms of iodine per day for each dog. This readily supplied the average daily iodine requirement of an adult dog, of 33 micrograms per kilo.[3]

Carrier-free radioactive iodine (I^{131}) having a physical half-life of eight days, was used in this study. This material was obtained both from the Radioisotope Laboratory at the University of Alberta Hospital, and from various commercial sources in Eastern Canada.

The arbitrary, standard dose of radioiodine used for these tracer studies was 4 microcuries per kilogram body weight of each animal. This compares with the average tracer dose of 50 microcuries per patient used in clinical diagnostic studies of thyroid function.[46]

Each quantity of radioiodine solution, when initially prepared, contained 100 microcuries of I^{131} per millilitre in distilled water. Daily decay was compensated by calculation from a standard table of the I^{131} disintegration curve.[46] Solutions prepared more than 8 days previous to any injection, were not used. The initial concentrated solution was carefully pipetted prior to use, and quantitatively diluted in distilled

water, so that one millilitre contained 4 microcuries of radioiodine. The required dose (4 microcuries per kilo), was then withdrawn from this solution into a syringe for injection.

In animals of Group I, following cannulation of the thoracic duct and jugular vein, the abdomen was opened through a midline incision. The stomach was identified and grasped with forceps. The dose of radioiodine was injected into the stomach by an associate. Presence of the needle in the stomach was confirmed by withdrawal of air or milky fluid into the syringe just prior to injection. The needle and syringe were withdrawn, care being taken not to contaminate instruments, surrounding tissues, or the operator with residual radioactivity in the syringe. The stomach was released and the abdomen closed (Figure II). This operative abdominal procedure also served as a control laparotomy for animals in Group II, in whom the common bile duct was cannulated.

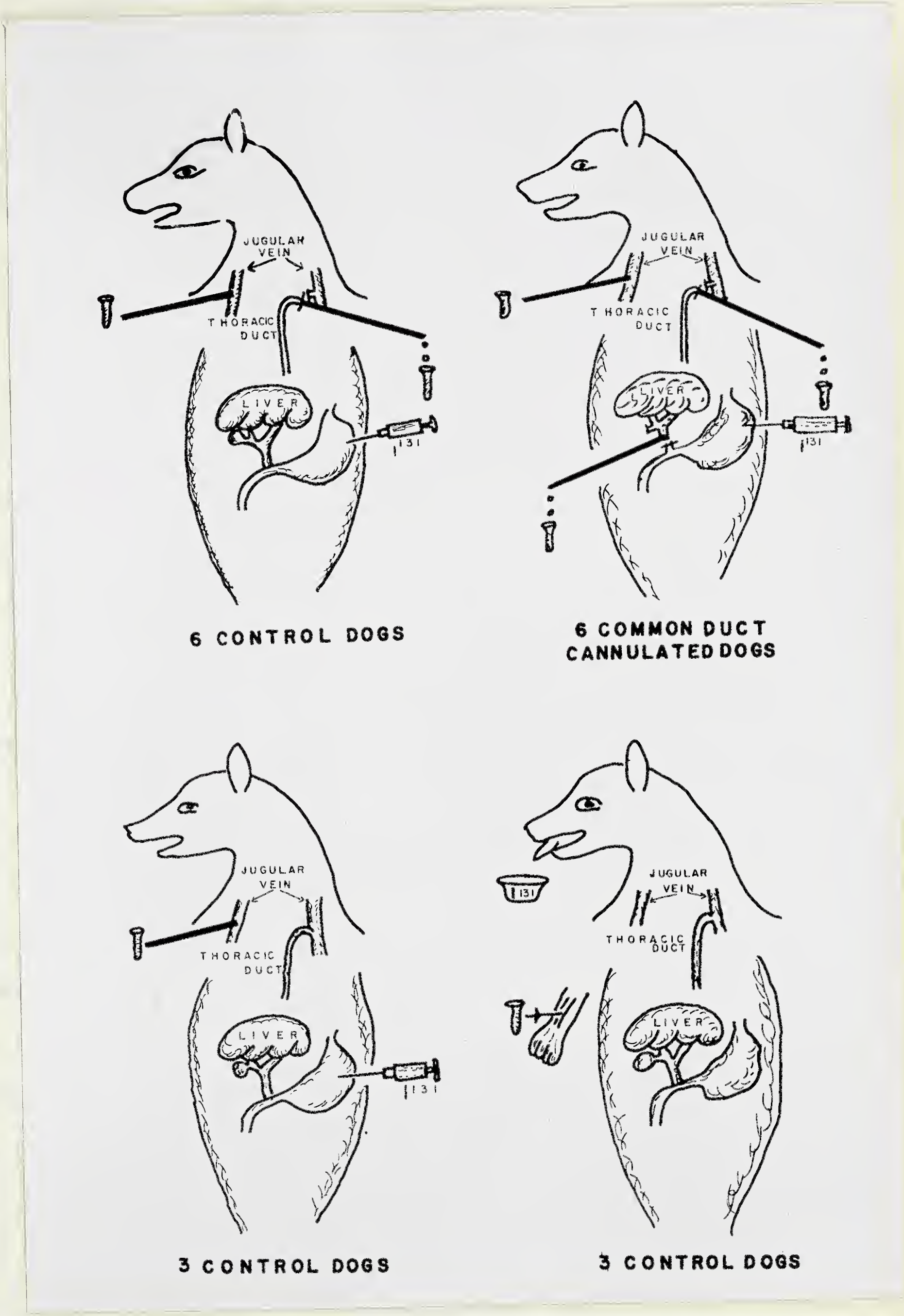
In animals of Group II, following the cannulation procedures of thoracic duct and jugular vein, radioiodine was similarly injected into the stomach while the abdomen was open immediately following cannulation of the common bile duct. The abdomen was then closed (Figure II).

In animals of Group III, following cannulation of the jugular vein, the abdomen was entered through a midline incision and radioiodine was injected into the stomach in a similar fashion (Figure II).

In animals of Group IV, in whom no anesthesia or operative procedure was carried out, the dose of radioiodine was placed in a clean feeding bowl, diluted with about 50 mls. of distilled water, and ingested by the animal. Each animal was isolated to avoid contamination by its secretions (Figure II).

FIGURE II

Group I Group II



6 CONTROL DOGS

6 COMMON DUCT CANNULATED DOGS

3 CONTROL DOGS

3 CONTROL DOGS

Group III Group IV

PART C

Collection of Samples

To determine the appearance and progression of radioactivity in blood and lymph, samples were taken at frequent intervals during a 12 hour period following the administration of radioiodine.

In animals in Groups I and II, collection intervals were as follows: 10 minute intervals for the first one-half hour, half-hourly for the first 4 hours, then hourly for the remainder of the 12 hours. In 3 animals in Group II, bile was collected following radioiodine injection. Collection of bile was divided into the following intervals: half-hourly for the first 4 hours, then hourly for the remainder of the 12 hours.

Control animals in Groups III and IV had samples of blood taken at intervals of only 1, 4 and 12 hours following administration of radioiodine.

Blood was collected in heparinized, vacuum tubes. Samples were readily obtained from animals in Groups I, II, and III in whom the jugular veins were cannulated. At the required intervals, the dilute heparin solution in the polythene tubes was removed, and about 5 mls. of venous blood withdrawn into the vacuum tubes. In animals in Group IV, at the required intervals, about 5 mls. of blood was withdrawn into vacuum tubes from the cubital vein in the foreleg.

Within an hour following removal, each blood sample was centrifuged for 15 minutes at 3000 revolutions per minute (r.p.m.), effectively

separating cells and plasma. Hemolysis was minimal. One millilitre aliquot samples of plasma were pipetted into clean dry test tubes for counting of radioactivity.

The total lymph drainage during 12 hours from the cannulated thoracic duct was collected from each dog in Groups I and II. The collection period was divided into the intervals previously described. Lymph was collected in heparinized, graduated centrifuge tubes. The volume in each tube was noted, and the collected sample was centrifuged for 15 minutes at 3000 r.p.m. to separate any erythrocytes from the lymph. One millilitre aliquot samples of lymph were pipetted into clean, dry test tubes for counting of radioactivity.

The total bile drainage during 12 hours from the cannulated common bile duct was collected from each of three dogs in Group II. The collection period was divided into the intervals previously described. Bile was collected in graduated test tubes. The volume in each tube was noted, and one millilitre aliquot samples of bile were pipetted into clean, dry test tubes for counting of radioactivity.

PART D

Counting and Calculation of Radioactivity

Radioactivity was counted in a Model 810-A Well Scintillation Detector connected to a Tracerlab Superscaler SC-18. This Well detector permitted the direct counting of each millilitre sample of fluid in a test tube.

A standard solution was prepared by diluting 2 millilitres (containing 8 microcuries of radioiodine), of the original solution used for each injection in 100 mls. of distilled water. One ml. of this diluted solution pipetted into a clean, dry test tube served as a standard for the counting of radioactivity of samples from each animal.

The one ml. aliquots of plasma, lymph or bile were counted for 3 minutes, and the count per minute (c.p.m.) was determined. The standard solution was counted at the same time, and counts of this standard solution and of background activity were repeated at frequent intervals to insure stability of the counting of the samples. The corrected count per minute of each one ml. aliquot sample was determined by subtracting the background count from the apparent count.

A sample counting follows:

Group No.:	I
Dog No.:	3
Sample:	Plasma
Time:	1 Hour
Apparent count per 3 minutes of sample:	4500
Apparent count per 1 minute of sample:	1500

Background count per 1 minute:	253
Corrected count per 1 minute of sample:	1247

The radioactivity in counts per minute of each injected dose of radioiodine was determined from the corrected count per minute of the standard solution. Each one ml. aliquot of standard solution represented a dilution of the same initial solution that was administered to each animal. The radioactivity of the injected dose could thus be determined by multiplication of the c.p.m. of the standard by the diluting factor and by the number of millilitres of the injected dose.

A sample calculation follows:

Group No.:	II
Dog No.:	8
Sample:	Standard solution
Corrected count per minute of sample:	21,119
Weight of dog:	15 kilos
Injected Dose (4 microcuries per kilo):	60 microcuries
Injected Dose (4 microcuries per ml.):	15 mls.
Diluted Standard Solution:	2 mls. (8 microcuries) of injected solution in 100 mls. water.
Corrected count per minute of 1 ml. aliquot of diluted standard:	21,119
Diluting factor (100/8):	12.5
Count per minute of 1 microcurie: (21,119 x 12.5)	263,987
Count per minute of injected dose: (60 microcuries): 263,987 x 60 :	15,839,220

For purposes of comparison, radioactivity of the samples was expressed as a percentage of the injected dose. Calculation of this

percentage for the one ml. samples of plasma, lymph or bile produced a fraction of one per cent. For purposes of presentation, this was converted to a per cent of the injected dose per litre.

The concentrations of radioactivity of the one ml. aliquot samples of plasma and lymph collected during the 12 hour period following radioiodine injection in animals in Groups I and II were thus calculated and expressed as a percentage of the injected dose per litre.

In addition, radioactivity of the hourly output of lymph in each animal in Groups I and II was calculated by multiplying the radioactivity of one ml. of each hourly sample by the volume (in mls.) of the sample. Total radioactivity of all the lymph collected during the 12 hour period was then determined in each dog of Groups I and II by summation of the calculated hourly radioactivity of the lymph.

Concentrations of radioactivity of the one ml. samples of bile were similarly calculated as described, and expressed as a percentage of the injected dose per litre, in the 3 animals of Group II in whom bile was collected.

Concentrations of radioactivity of the one ml. samples of plasma in animals of Groups III and IV were also similarly calculated, and presented as a percentage of the injected dose per litre.

CHAPTER III

PRESENTATION OF RESULTS

Results are presented in the Tables and Figures that follow.

The volume of lymph drainage from the thoracic ducts in the 6 control dogs comprising Group I, is presented in Table II A. The average hourly excretion of lymph is relatively stable in each animal. A tendency to greatest excretion appears in the 4 to 6 hour period, while the quantity per hour tended to decrease in the 12 hour period in each animal.

Table II B presents the volume of lymph drainage collected from the thoracic ducts of the common bile duct cannulated dogs comprising Group II. The quantities of lymph excreted in these animals closely parallel the results obtained in Group I. Similarity occurs both in actual quantities of lymph excreted, and in the tendency for the highest excretion period to be at 4 to 6 hours, with a diminution in excretion during the latter part of the collection period.

The mean volumes and ranges of lymph excretion in animals of Groups I and II are presented in Table II C. A close parallel is apparent in the mean volumes of both groups during the 12 hour period.

In Table II D are presented the total lymph volumes excreted from each dog in both groups during 12 hours. There is less than 10 mls. difference in the mean volumes excreted in both groups of animals.

Standard deviations[23] were calculated using the following formula:

$$\text{Sample Variance} = \frac{(\text{Sum of each observation})^2}{\text{No. of observations}} - (\text{Average of observations})^2$$

$$\text{Standard Deviation} = \sqrt{\text{Sample Variance}}$$

Probability values were determined from standard tables of 't'.[23] Values of 't' were calculated using the following formula:

$$'t' = \frac{M_1 - M_2}{\sqrt{\frac{N_1S_1 + N_2S_2}{N_1 + N_2 - 2} \times \frac{N_1 + N_2}{N_1\ N_2}}}$$

Sample -1: M_1 = mean

N_1 = number

S_1 = sample variance

Sample -2: M_2 = mean

N_2 = number

S_2 = sample variance

TABLE II A

VOLUME (IN MLS.) OF LYMPH DRAINAGE FROM THE THORACIC DUCT IN 6 CONTROL DOGS

TIME IN HOURS	GROUP I DOG NO. 1	2	3	4	5	6
1/6	1.8	2.5	2.2	2.6	2.0	3.4
1/3	1.2	3.0	2.3	1.6	1.0	3.0
1/2	1.2	1.7	2.3	2.6	1.0	2.7
1	8.5	4.0	9.0	5.3	3.0	5.9
1.5	10.0	4.5	8.8	2.3	4.5	13.9
2	10.0	3.2	8.4	5.0	7.4	12.6
2.5	9.0	5.0	11.6	5.1	7.2	13.5
3	9.5	4.0	7.3	5.0	7.3	12.9
3.5	9.0	3.0	6.0	4.4	7.5	11.2
4	9.5	2.7	5.8	6.8	6.9	12.1
5	19.0	4.8	16.5	10.0	15.8	35.0
6	20.5	4.5	16.8	12.0	15.0	28.0
7	18.0	4.5	14.0	16.0	13.0	27.0
8	16.0	4.7	12.0	13.5	11.8	20.0
9	14.0	7.5	10.5	15.0	8.5	18.0
10	13.0	5.5	9.5	17.4	9.0	19.0
11	16.5	6.8	9.0	11.0	6.0	18.0
12	15.0	5.0	6.6	13.5	4.6	15.0

TABLE II B

VOLUME (IN MLS.) OF LYMPH DRAINAGE FROM THE THORACIC DUCT IN 6 COMMON BILE DUCT CANNULATED DOGS

TIME IN HOURS	GROUP II DOG NO.					
	7	8	9	10	11	12
1/6	3.5	2.8	1.4	4.0	1.2	3.4
1/3	3.5	3.5	1.3	5.1	0.2	3.6
1/2	2.6	4.1	1.4	5.0	0.7	3.5
1	9.0	10.5	3.6	11.2	3.9	10.0
1.5	6.0	7.0	3.7	8.3	5.1	10.9
2	5.5	7.5	4.8	9.5	5.6	9.4
2.5	6.5	6.5	5.3	10.7	2.3	11.7
3	5.0	6.5	5.1	8.4	4.5	10.6
3.5	5.1	8.5	6.6	9.4	2.2	11.1
4	6.0	7.5	5.1	10.0	4.3	10.9
5	9.0	15.0	19.0	24.0	10.0	19.0
6	6.0	12.5	19.0	25.0	11.0	18.0
7	6.5	12.0	16.0	17.0	12.0	18.5
8	8.0	12.0	22.0	14.0	12.0	16.5
9	6.0	10.0	16.0	15.0	15.0	14.0
10	4.5	11.0	20.0	13.0	13.0	14.0
11	3.5	8.0	14.0	13.0	10.0	13.0
12	6.5	9.5	12.5	13.0	9.0	11.0

TABLE II C

MEAN VOLUME (IN MLS.) OF LYMPH DRAINAGE FROM THE THORACIC DUCT IN 6 CONTROL DOGS, AND IN 6 COMMON BILE DUCT CANNULATED DOGS

TIME IN HOURS	CONTROL DOGS GROUP I		COMMON DUCT DOGS GROUP II	
	MEAN	RANGE	MEAN	RANGE
1/6	2.4	1.8 - 3.4	2.7	1.2 - 4.0
1/3	2.0	1.0 - 3.0	2.9	0.2 - 5.1
1/2	1.9	1.0 - 2.7	2.9	0.7 - 5.0
1	5.9	3.0 - 9.0	8.0	3.6 - 11.2
1.5	7.3	2.3 - 13.9	6.8	3.7 - 10.9
2	7.8	3.2 - 12.6	7.0	5.5 - 9.5
2.5	8.6	5.0 - 13.5	7.2	2.3 - 11.7
3	7.7	4.0 - 12.9	6.7	4.5 - 10.6
3.5	6.8	3.0 - 11.2	7.1	2.2 - 11.1
4	7.3	2.7 - 12.1	7.3	4.3 - 10.9
5	16.8	4.8 - 35.0	16.0	9.0 - 24.0
6	16.1	4.5 - 28.0	15.2	6.0 - 25.0
7	15.4	4.5 - 27.0	13.7	6.5 - 18.5
8	13.0	4.7 - 20.0	14.1	8.0 - 22.0
9	12.2	7.5 - 18.0	12.6	6.0 - 16.0
10	12.2	5.5 - 19.0	12.6	4.5 - 20.0
11	11.2	6.0 - 18.0	10.1	3.5 - 14.0
12	9.9	4.6 - 15.0	10.2	6.5 - 12.5

TABLE II D

TOTAL AND MEAN VOLUME OF LYMPH DRAINAGE (IN MLS.) FROM THE THORACIC DUCT DURING 12 HOURS IN 6 CONTROL DOGS, AND 6 COMMON BILE DUCT CANNULATED DOGS

CONTROL DOGS GROUP I DOG NO.		COMMON DUCT DOGS GROUP II DOG NO.	
1	201.7	7	162.9
2	76.9	8	154.4
3	158.6	9	176.8
4	149.1	10	215.6
5	131.5	11	122.0
6	271.2	12	209.1
MEAN	164.8	MEAN	173.4
*S.D.	±66	*S.D.	±35
RANGE	76.9 - 271.2	RANGE	122.0 - 215.6
PROBABILITY	> 0.45 (Insignificant)		

*STANDARD DEVIATION

The concentration of radioactivity of each ml. aliquot sample of plasma collected at intervals during 12 hours was calculated as a percentage of the injected dose per litre. Table III A presents these results in the control dogs of Group I. In most dogs, radioactivity is insignificant until one hour following radioiodine injection.

The concentration of radioactivity of each ml. aliquot sample of lymph collected at intervals during 12 hours was calculated as a percentage of the injected dose per litre. Table III B presents these results in the control dogs of Group I. These results are similar to those in Table III A.

Table III C presents the concentrations of radioactivity in the common bile duct cannulated dogs of Group II. Values obtained in these animals are considerably lower than those in Group I animals reported in Table III A.

Table III D presents the concentrations of radioactivity in lymph in Group II animals. These results parallel the plasma concentrations in Table III C. The values are also considerably lower than those apparent in Group I animals in Table III B.

Mean concentrations and ranges of radioactivity in plasma in dogs of Groups I and II are compared in Table III E. The mean values in Group I are considerably higher than those of Group II. Mean radioactivity in Group I animals tends to decrease by the end of the 12 hour period, while that of Group II animals appears to be increasing, or reaching a plateau level.

Table III F presents the mean concentrations and ranges of radioactivity in lymph in animals of Groups I and II. Mean values in Group I animals are considerably higher than the mean values obtained in Group II. Radioactivity in Group I animals appears to be decreasing by the end of the collection period, while that of Group II dogs is increasing, or reaching a plateau.

TABLE III A

CONCENTRATION OF RADIOACTIVITY IN PLASMA IN 6 CONTROL DOGS EXPRESSED AS A PER CENT OF THE INJECTED DOSE PER LITRE

TIME IN HOURS	GROUP I DOG NO.					
	1	2	3	4	5	6
1/6	0	.76	.64	.60	2.77	.01
1/3	.12	7.19	.60	.85	7.21	.12
1/2	0	8.90	.71	.88	8.76	4.91
1	15.24	17.99	16.00	9.90	25.60	10.06
1.5	20.35	23.68	20.98	13.80	24.87	13.96
2	16.40	22.40	22.29	16.22	24.85	15.28
2.5	15.69	22.28	22.42	19.65	26.05	14.58
3	14.70	20.80	20.98	18.52	26.45	15.31
3.5	14.91	21.60	21.81	18.33	26.20	15.68
4	13.37	19.19	20.01	18.83	28.65	15.26
5	13.81	19.19	24.22	18.01	25.40	15.18
6	14.37	22.26	22.01	17.17	24.14	15.21
7	14.50	18.83	20.36	15.37	22.02	15.43
8	13.52	19.77	20.75	15.00	20.92	15.27
9	13.64	19.43	16.99	13.58	20.74	14.75
10	14.92	23.06	16.62	13.17	21.18	14.70
11	13.81	18.13	15.75	13.31	21.56	14.65
12	13.29	18.15	15.90	13.11	19.27	13.59

TABLE III B

CONCENTRATION OF RADIOACTIVITY IN LYMPH IN 6 CONTROL DOGS
EXPRESSED AS A PER CENT OF THE INJECTED DOSE PER LITRE

TIME IN HOURS	GROUP I DOG NO.					
	1	2	3	4	5	6
1/6	.23	.86	.42	0	.27	0
1/3	.23	.66	.40	.41	.66	.04
1/2	0	1.58	.63	.47	7.34	.05
1	.77	19.96	11.37	11.35	12.01	2.29
1.5	11.26	21.50	12.16	12.07	27.47	11.25
2	17.55	25.97	15.19	15.03	26.10	13.87
2.5	17.26	29.89	15.43	18.08	29.81	15.31
3	16.13	28.79	19.34	19.81	29.04	15.70
3.5	14.74	26.87	18.76	22.16	30.88	15.88
4	14.64	24.28	18.61	21.38	28.68	16.10
5	13.79	24.14	20.40	22.05	28.28	15.40
6	14.68	25.54	25.08	21.06	28.41	16.20
7	14.61	24.42	20.80	19.84	26.14	16.08
8	13.60	24.42	19.48	16.69	23.38	16.17
9	13.70	24.66	19.01	16.38	21.47	16.14
10	15.24	24.22	17.97	16.07	21.20	16.23
11	16.15	21.62	16.81	15.28	21.47	15.29
12	15.90	20.16	15.32	14.96	20.71	14.44

TABLE III C

CONCENTRATION OF RADIOACTIVITY IN PLASMA IN 6 COMMON BILE DUCT CANNULATED DOGS, EXPRESSED AS A PER CENT OF THE INJECTED DOSE PER LITRE

TIME IN HOURS	GROUP II DOG NO.					
	7	8	9	10	11	12
1/6	0	.176	1.766	.016	.009	.028
1/3	.465	.151	0	.101	.514	.720
1/2	.980	.453	1.947	.391	.156	1.063
1	1.837	.585	3.124	.430	.606	1.007
1.5	1.915	.824	1.947	.516	.533	1.042
2	2.133	.736	2.581	.766	.551	1.182
2.5	2.245	.780	3.441	1.058	.744	1.199
3	2.800	.956	2.988	1.082	.997	1.231
3.5	3.382	1.510	4.075	1.277	1.011	1.329
4	2.438	1.485	4.528	1.949	1.102	1.346
5	3.774	1.460	6.701	2.070	6.394	1.371
6	4.317	2.089	4.346	2.144	6.587	1.364
7	4.345	1.592	4.618	2.316	5.889	2.105
8	9.525	1.831	6.067	2.433	7.708	1.913
9	8.730	1.951	6.610	2.355	7.111	1.937
10	9.637	2.347	6.791	2.043	6.606	2.133
11	9.699	2.177	6.972	1.965	5.999	2.168
12	10.516	2.177	5.886	1.969	4.520	2.448

TABLE III D

CONCENTRATION OF RADIOACTIVITY IN LYMPH IN 6 COMMON BILE DUCT CANNULATED DOGS, EXPRESSED AS A PER CENT OF THE INJECTED DOSE PER LITRE						
TIME IN HOURS	GROUP II DOG NO.					
	7	8	9	10	11	12
1/6	.106	0	0	0	.129	.024
1/3	.420	.025	0	0	.230	.091
1/2	.308	0	2.083	.422	0560	.385
1	1.047	.321	1.811	.422	.367	.469
1.5	1.747	.774	0	.437	.735	.801
2	2.033	.604	1.811	.867	.854	.937
2.5	2.469	.717	.181	.781	.666	1.000
3	2.845	.831	1.720	.922	.942	1.056
3.5	2.822	1.007	2.264	.851	1.070	1.112
4	3.881	1.365	2.988	1.000	.942	1.108
5	4.345	1.825	4.799	1.269	5.085	1.112
6	4.463	1.844	6.519	1.281	6.261	1.161
7	4.917	2.315	8.331	1.301	7.901	1.469
8	4.973	2.548	6.519	1.262	7.258	1.587
9	8.724	2.347	7.425	1.301	8.177	1.633
10	9.620	2.473	5.705	1.254	6.156	1.612
11	10.124	2.460	6.519	1.246	6.201	1.797
12	10.242	2.272	4.709	1.172	1.864	1.864

TABLE III E

MEAN CONCENTRATION OF RADIOACTIVITY IN PLASMA IN 6 CONTROL DOGS AND 6 COMMON BILE DUCT CANNULATED DOGS, EXPRESSED AS A PER CENT OF THE INJECTED DOSE PER LITRE

TIME IN HOURS	CONTROL DOGS GROUP I		COMMON DUCT DOGS GROUP II	
	MEAN	RANGE	MEAN	RANGE
1/6	.80	0 - 2.77	.332	0 - 1.766
1/3	2.68	.12 - 7.21	.325	0 - .720
1/2	4.03	0 - 8.90	.832	.156 - 1.947
1	15.80	9.90 - 25.60	1.265	.430 - 3.124
1.5	19.60	13.80 - 24.87	1.129	.516 - 1.947
2	19.57	15.28 - 24.35	1.325	.551 - 2.581
2.5	20.11	14.58 - 26.05	1.578	.744 - 3.441
3	19.46	14.70 - 26.45	1.676	.956 - 2.988
3.5	19.75	14.91 - 26.20	2.097	1.011 - 4.075
4	19.22	13.37 - 28.65	2.308	1.102 - 4.528
5	19.30	13.81 - 25.40	3.628	1.371 - 6.701
6	19.19	14.37 - 24.14	3.474	1.364 - 6.587
7	17.75	14.50 - 22.02	3.477	1.592 - 5.389
8	17.54	13.52 - 20.92	4.913	1.831 - 9.525
9	16.52	13.58 - 20.74	4.782	1.937 - 8.730
10	17.27	13.17 - 23.06	4.926	2.043 - 9.637
11	16.20	13.31 - 21.56	4.830	1.965 - 9.699
12	15.55	13.11 - 19.27	4.586	1.969 - 10.516

TABLE III F

MEAN CONCENTRATION OF RADIOACTIVITY IN LYMPH IN 6 CONTROL DOGS AND 6 COMMON BILE DUCT CANNULATED DOGS, EXPRESSED AS A PER CENT OF THE INJECTED DOSE PER LITRE

TIME IN HOURS	CONTROL DOGS GROUP I		COMMON DUCT DOGS GROUP II	
	MEAN	RANGE	MEAN	RANGE
1/6	.30	0 - .86	.043	0 - .129
1/3	.80	.04 - .66	.127	0 - .420
1/2	1.68	0 - 7.34	.626	0 - 2.083
1	9.62	.77 - 19.96	.739	.321 - 1.811
1.5	15.95	11.25 - 27.47	.749	0 - 1.747
2	18.95	13.87 - 26.10	1.184	.604 - 2.033
2.5	20.96	15.31 - 29.39	.969	.666 - 2.469
3	21.47	15.70 - 29.04	1.386	.831 - 2.845
3.5	21.55	14.74 - 30.88	1.521	.851 - 2.822
4	20.61	14.64 - 28.68	1.831	.942 - 3.881
5	20.68	13.79 - 28.28	3.072	1.112 - 5.085
6	21.83	14.68 - 28.41	3.588	1.161 - 6.519
7	20.31	14.61 - 26.14	4.372	1.301 - 8.331
8	18.96	13.60 - 24.42	4.024	1.262 - 7.258
9	18.56	13.70 - 24.66	4.934	1.301 - 8.724
10	18.49	15.24 - 24.22	4.470	1.254 - 9.620
11	17.77	15.28 - 21.62	4.724	1.246 - 10.124
12	16.91	14.44 - 20.71	4.264	1.172 - 10.242

Table III G presents a synthesis of the results obtained in the previous tables of concentrations of radioactivity in plasma and lymph in animals of Groups I and II. Figure III portrays these results graphically. The concentrations of radioactivity in plasma and lymph are very similar in each group during the collection period, with a marked divergence between the two groups. A lower concentration of radioactivity in both plasma and lymph is present in the common bile duct cannulated dogs of Group II than in the control animals of Group I.

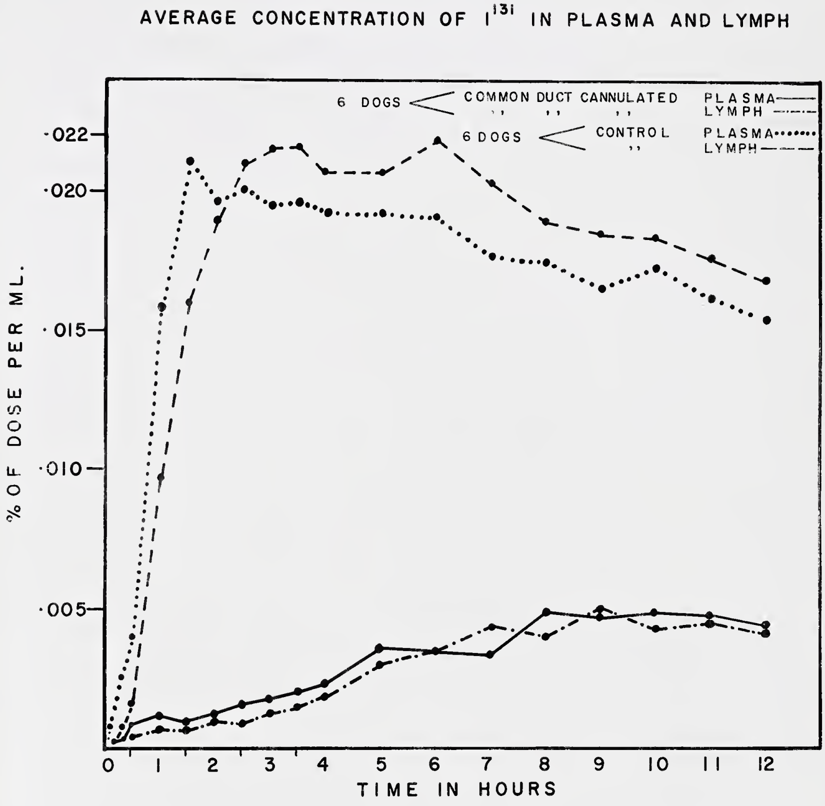
The highest concentrations of radioactivity in both lymph and plasma in animals in Group I occur in the 2 to 6 hour range, while the highest values in the animals in Group II appear during the 8 to 12 hour period.

TABLE III G

AVERAGE CONCENTRATION OF I 131 IN PLASMA AND LYMPH
AS % OF DOSE PER LITRE

GROUP	ONE ML. SAMPLES	TIME IN HOURS											
		1	2	3	4	5	6	7	8	9	10	11	12
6 CONTROL DOGS	PLASMA	15.8	19.6	19.5	19.2	19.3	19.2	17.7	17.5	16.5	17.3	16.2	15.5
	LYMPH	9.6	18.9	21.5	20.6	20.7	21.8	20.3	18.9	18.5	18.5	17.8	16.9
6 BILE DUCT CANNULATED DOGS	PLASMA	1.3	1.3	1.7	2.3	3.6	3.5	3.5	4.9	4.8	4.9	4.8	4.6
	LYMPH	0.7	1.1	1.4	1.9	3.1	3.6	4.4	4.0	4.9	4.5	4.7	4.3

FIGURE III


AVERAGE CONCENTRATION OF I131 IN PLASMA AND LYMPH
6 DOGS
COMMON DUCT CANNULATED
PLASMA
LYMPH
6 DOGS
CONTROL
PLASMA
LYMPH
% OF DOSE PER ML.
·022
·020
·015
·010
·005
0
1
2
3
4
5
6
7
8
9
10
11
12
TIME IN HOURS

Radioactivity of the hourly output of lymph in each animal in Group I is presented in Table IV A. By the second hour of collection in each animal, a high level of radioactivity appears.

Table IV B presents the radioactivity of the hourly output of lymph in each animal in Group II. The values obtained in these animals are considerably lower than those in Group I animals presented in Table IV A.

Mean radioactivity and ranges of the hourly lymph excretions in animals in Groups I and II are presented in Table IV C. Mean values in Group I animals are considerably higher than the mean values obtained in Group II.

Figure IV portrays graphically the results presented in Table IV C. The difference in radioactivity of the hourly lymph excretion between the two groups appears in all periods during the collection.

The greatest radioactivity occurs in the 3 to 7 hour period in animals in Group I, and during the 7 to 9 hour period in the animals in Group II.

TABLE IV A

RADIOACTIVITY OF THE HOURLY LYMPH OUTPUT IN 6 CONTROL DOGS, EXPRESSED AS A PER CENT OF THE INJECTED DOSE PER LITRE

TIME IN HOURS	GROUP I DOG NO.					
	1	2	3	4	5	6
1	7.3	50.7	105.6	62.0	44.5	13.8
2	288.3	179.8	234.6	102.9	316.7	331.1
3	308.6	264.6	320.2	191.2	426.6	409.2
4	271.7	146.2	220.5	242.9	429.5	372.7
5	262.1	115.9	336.6	220.6	446.8	539.2
6	300.9	110.4	421.4	252.8	426.2	453.5
7	263.0	109.9	291.2	317.4	339.8	434.1
8	217.6	114.8	233.8	225.4	275.9	323.4
9	191.8	185.0	199.6	245.7	182.5	290.5
10	198.1	133.2	170.7	279.5	190.8	308.4
11	266.5	147.0	151.3	168.1	128.8	275.3
12	238.6	100.8	101.1	202.0	95.2	216.6

TABLE IV B

RADIOACTIVITY OF THE HOURLY LYMPH OUTPUT IN 6 DOGS WITH CANNULATED COMMON BILE DUCTS, EXPRESSED AS A PER CENT OF THE INJECTED DOSE PER LITRE

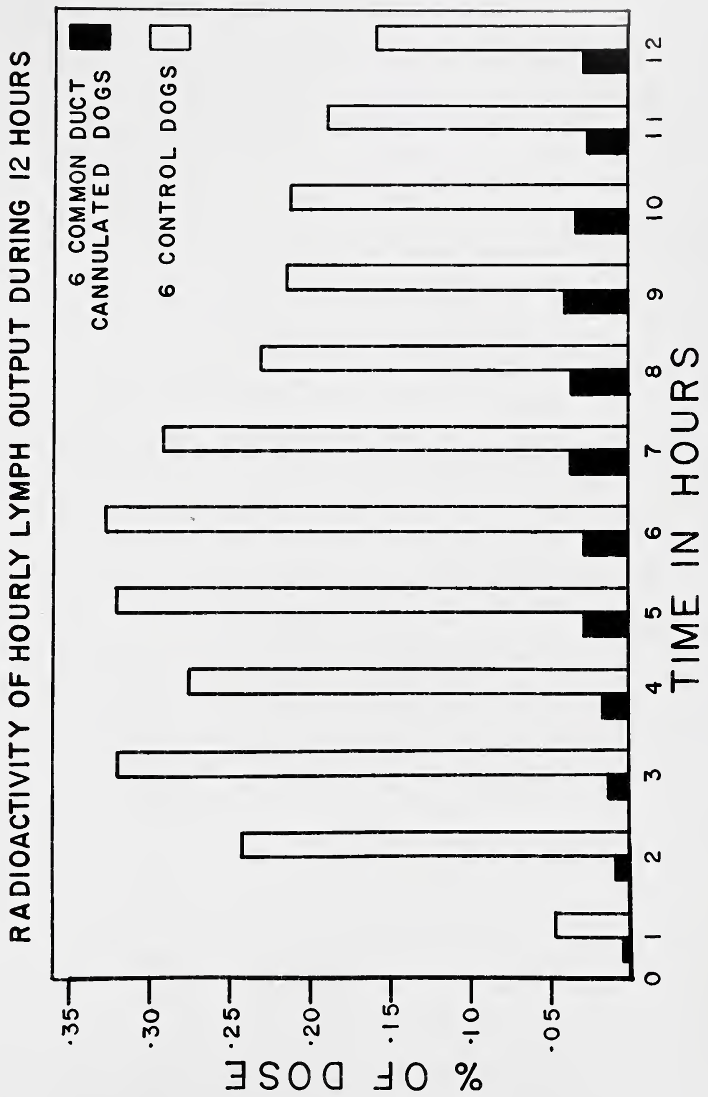
TIME IN HOURS	GROUP II DOG NO.					
	7	8	9	10	11	12
1	11.8	3.4	.9	6.8	2.4	6.4
2	21.7	9.9	.9	11.9	8.5	17.5
3	30.3	10.1	.9	16.1	5.8	22.9
4	37.7	18.8	3.0	18.0	6.4	24.4
5	39.1	27.4	9.1	30.5	50.8	21.1
6	26.7	23.0	12.4	32.0	68.9	20.9
7	31.9	27.8	13.3	22.1	94.8	27.2
8	39.8	30.6	14.3	17.7	87.0	26.2
9	52.3	23.5	11.9	19.5	122.6	22.9
10	43.2	27.2	11.4	16.3	78.8	22.6
11	35.4	19.7	9.1	16.2	62.0	23.3
12	66.5	21.6	5.9	15.2	47.9	20.5

TABLE IV C

MEAN RADIOACTIVITY OF THE HOURLY LYMPH OUTPUT IN 6 CONTROL DOGS, AND IN 6 DOGS WITH CANNULATED COMMON BILE DUCTS, EXPRESSED AS A PER CENT OF THE INJECTED DOSE PER LITRE

TIME IN HOURS	CONTROL DOGS GROUP I		COMMON DUCT DOGS GROUP II	
	MEAN	RANGE	MEAN	RANGE
1	47.3	7.3 - 105.6	5.3	.9 - 11.8
2	242.2	102.9 - 331.1	11.7	.9 - 21.7
3	320.1	191.2 - 426.6	14.3	.9 - 30.3
4	277.2	146.2 - 429.5	18.0	3.0 - 37.7
5	320.2	115.9 - 539.2	29.7	9.1 - 50.8
6	327.5	110.4 - 453.5	30.6	12.4 - 68.9
7	292.6	109.9 - 434.1	36.2	13.3 - 94.8
8	231.8	114.8 - 323.4	35.9	14.3 - 87.0
9	215.3	185.0 - 290.5	42.1	11.9 - 122.6
10	213.4	133.2 - 308.4	33.2	11.4 - 78.8
11	189.5	147.0 - 275.3	25.9	9.1 - 62.0
12	159.0	100.8 - 216.6	29.6	5.9 - 66.5

FIGURE IV



RADIOACTIVITY OF HOURLY LYMPH OUTPUT DURING 12 HOURS

Table V presents the total radioactivity of lymph drainage during the 12 hour period in each control animal in Group I and in each common bile duct cannulated dog in Group II. Means, ranges, and standard deviations of these values are also presented. Radioactivity in Group I dogs is uniformly higher than in Group II animals.

Table VI presents a synthesis of the mean results of total lymph volume and total radioactivity of the lymph volume from the animals in Groups I and II. While the volumes of lymph produced in each group of dogs are nearly identical, the mean radioactivity of the lymph from control animals in Group I is nearly 10 times as great as the mean radioactivity of the lymph from the common bile duct cannulated dogs in Group II. There is no overlapping of the ranges.

TABLE V

TOTAL AND MEAN RADIOACTIVITY OF THE LYMPH DRAINAGE DURING 12 HOURS IN 6 CONTROL DOGS, AND 6 DOGS WITH CANNULATED COMMON BILE DUCTS, EXPRESSED AS A PER CENT OF THE INJECTED DOSE

CONTROL DOGS GROUP I		COMMON DUCT DOGS GROUP II	
DOG NO.		DOG NO.	
1	2.81	7	0.44
2	1.66	8	0.24
3	2.79	9	0.09
4	2.51	10	0.22
5	3.30	11	0.64
6	3.97	12	0.25
MEAN	2.84	MEAN	0.31
*S.D.	-0.77	*S.D.	-0.19
RANGE	1.66 - 3.97	RANGE	0.09 - 0.64
PROBABILITY	< 0.005 (Highly Significant)		

*STANDARD DEVIATION

TABLE VI

TOTAL VOLUME AND RADIOACTIVITY OF LYMPH DURING 12 HOURS

GROUP		VOLUME IN ML.	RADIOACTIVITY IN % OF DOSE
6 CONTROL DOGS	MEAN	165	2.84
	S.D.	± 66	± 0.77
	RANGE	77 - 271	1.66 - 3.97
6 BILE DUCT CANNULATED DOGS	MEAN	173	0.31
	S.D.	± 35	± 0.19
	RANGE	122 - 216	0.09 - 0.64

Table VII presents the concentrations of radioactivity in plasma in the control dogs comprising Groups III and IV. In these animals the highest concentrations appear in the 1 or 4 hour samples, with a decrease in the 12 hour samples. Individual values of each interval among the various dogs of both groups are comparable.

Table VIII presents the mean concentrations of radioactivity in the plasma in all 4 groups of dogs, at 1, 4 and 12 hours. The values in Group II animals are considerably lower than those in the remaining groups.

Figure V portrays graphically the results presented in Table VIII. Results of mean plasma samples at only 1, 4 and 12 hours are used to make the comparison more valid. Line A represents the mean values from the six control dogs in Group I. Line B represents values from the three physiological control dogs in Group IV. Line C represents values from the three dogs in Group III in whom minimal procedures were done. Line D represents values from the six common bile duct cannulated dogs in Group II.

Concentrations of radioactivity are similar among the animals in Groups I, III and IV, with a tendency to decreasing values in the final samples. Radioactivity of plasma samples in Group II animals, however, is considerably lower with an increase occurring during the 4 to 12 hour period.

TABLE VII

CONCENTRATION OF RADIOACTIVITY IN PLASMA IN 2 FURTHER GROUPS OF CONTROL DOGS, EXPRESSED AS A PER CENT OF THE INJECTED DOSE PER LITRE

TIME IN HOURS	GROUP III DOG NO.			GROUP IV DOG. NO.		
	13	14	15	16	17	18
1	25.18	15.45	7.19	16.90	13.06	11.07
4	16.49	14.07	14.24	19.04	15.10	12.56
12	15.15	12.42	11.32	17.69	14.33	12.41

TABLE VIII

MEAN CONCENTRATION OF RADIOACTIVITY IN PLASMA IN ALL 4 GROUPS OF DOGS, AT 1, 4, AND 12 HOURS, EXPRESSED AS A PER CENT OF THE INJECTED DOSE PER LITRE

TIME IN HOURS	GROUP I (6 DOGS)	GROUP II (6 DOGS)	GROUP III (3 DOGS)	GROUP IV (3 DOGS)
1	15.80	1.265	15.94	13.68
4	19.22	2.308	14.93	15.57
12	15.55	4.586	12.96	14.81

FIGURE V

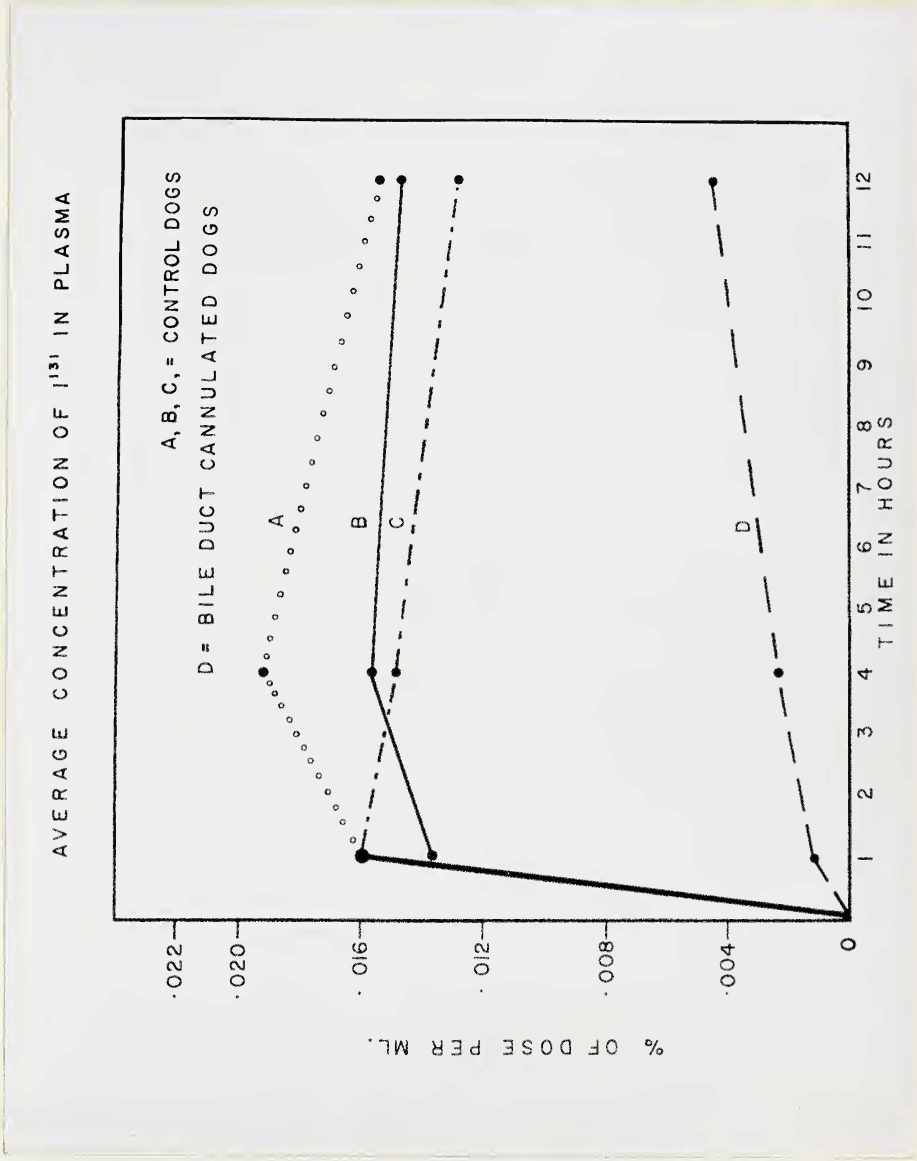

AVERAGE CONCENTRATION OF I^{131} IN PLASMA
A, B, C, = CONTROL DOGS
D = BILE DUCT CANNULATED DOGS
A
B
C
D
.022
.020
.016
.012
.008
.004
0
% OF DOSE PER ML.
1
2
3
4
5
6
7
8
9
10
11
12
TIME IN HOURS

Table IX A presents the individual and mean volumes of bile drainage collected from 3 animals with cannulated common bile ducts in Group II. Bile drainage is most profuse during the initial period, with a tendency to decreasing volume during the last 4 hours of the collection period. Somewhat higher values are present in dog No. 11 than in the other 2 animals.

Table IX B presents the total and mean volumes of bile drainage from the 3 animals in Group II in whom bile was collected. The total volume of bile collected in dog No. 11 is greater than that obtained from the remaining 2 animals.

TABLE IX A

VOLUME (IN MLS.) OF BILE DRAINAGE IN 3 DOGS WITH CANNULATED COMMON BILE DUCTS

TIME IN HOURS	GROUP II DOG NO. 10	11	12	MEAN VOLUME
1/2	3.1	8.3	3.5	5.0
1	2.1	5.5	2.8	3.5
1.5	1.3	5.0	1.6	2.6
2	1.4	3.8	4.7	3.3
2.5	1.6	2.6	9.3	4.5
3	0.7	2.4	3.7	2.3
3.5	0.9	5.5	3.7	3.4
4	1.5	6.0	5.3	4.3
5	4.0	13.5	5.4	7.6
6	3.4	18.0	2.7	8.0
7	2.0	12.5	1.6	5.4
8	2.2	8.0	1.3	3.8
9	2.2	5.5	1.0	2.9
10	1.8	4.5	1.0	2.4
11	2.0	3.6	0.8	2.1
12	2.0	4.5	1.0	2.5

TABLE IX B

TOTAL AND MEAN VOLUME OF BILE DRAINAGE (IN MLS.) DURING 12 HOURS IN 3 DOGS WITH CANNULATED COMMON BILE DUCTS			
GROUP II DOG NO.			
10	11	12	MEAN
32.2	109.2	49.4	63.6

Table X A presents the concentrations of radioactivity in the one ml. samples of plasma, lymph and bile collected at intervals during 12 hours in 3 dogs in Group II in whom bile was collected. The concentrations of radioactivity of bile in each of these animals increases gradually until the 4 hour period, when it appears to reach a plateau. Values of the concentration of radioactivity of bile at each interval are comparable among the 3 animals, despite the variation in volume of bile collected that was noted in the two preceding tables. Values of the concentrations of radioactivity of lymph and plasma among the three animals are similar, with slightly higher results from dog No. 11 than the other two animals.

Table X B presents the mean concentrations and ranges of the values noted in Table X A. Mean concentrations of radioactivity of plasma and lymph are similar in these animals, while the mean values of radioactivity of bile are considerably higher.

Figure VI portrays graphically the results presented in Table X B. Concentration of radioactivity in lymph and plasma is nearly identical, with the highest values during the 8 to 10 hour period. Concentration of radioactivity of the bile increases sharply after 2 hours, with the peak values during the 7 to 11 hour period, and a tendency to decrease at the 12 hour level.

Table XI presents a comparison of the mean concentrations of radioactivity of plasma and lymph in the control dogs in Group I, and, of the mean concentrations of radioactivity in the bile in the 3 dogs in Group II in whom bile was collected. The values for plasma and lymph are higher at comparable intervals than are those for bile.

TABLE X A

CONCENTRATION OF RADIOACTIVITY IN PLASMA, LYMPH, AND BILE IN 3 DOGS WITH CANNULATED COMMON BILE DUCTS, EXPRESSED AS A PER CENT OF THE INJECTED DOSE PER LITRE

GROUP II

TIME IN HOURS	PLASMA			LYMPH			BILE		
	DOG NO.			DOG NO.			DOG NO.		
	10	11	12	10	11	12	10	11	12
1/6	.016	.009	.028	0	.129	.024	-	-	-
1/3	.101	.514	.720	0	.230	.091	-	-	-
1/2	.391	.156	1.063	.422	.560	.385	.125	.276	.741
1	.430	.606	1.007	.422	.367	.469	.930,	.487	2.049
1.5	.516	.533	1.042	.437	.735	.801	1.453	.671	2.049
2	.766	.551	1.182	.867	.854	.937	1.336	.735	2.084
2.5	1.058	.744	1.199	.781	.666	1.000	5.429	3.032	4.651
3	1.082	.997	1.231	.922	.942	1.056	5.328	7.120	5.490
3.5	1.277	1.011	1.329	.851	1.070	1.112	9.556	11.052	5.612
4	1.949	1.102	1.346	1.000	.942	1.108	9.957	10.763	5.563
5	2.070	6.394	1.371	1.269	5.085	1.112	13.417	11.650	7.116
6	2.144	6.587	1.364	1.281	6.261	1.161	12.691	13.000	8.829
7	2.316	5.889	2.105	1.301	7.901	1.469	12.339	14.746	9.378
8	2.433	7.708	1.913	1.262	7.258	1.587	11.207	12.578	12.029
9	2.355	7.111	1.937	1.301	8.177	1.633	10.828	14.002	11.882
10	2.043	6.606	2.133	1.254	6.156	1.612	11.023	12.481	12.253
11	1.965	5.999	2.168	1.246	6.201	1.797	10.687	11.700	14.882
12	1.969	4.520	2.448	1.172	5.329	1.864	10.664	9.661	14.393

TABLE X B

MEAN CONCENTRATION OF RADIOACTIVITY IN PLASMA, LYMPH, AND BILE, IN 3 DOGS WITH CANNULATED COMMON BILE DUCTS, EXPRESSED AS A PER CENT OF THE INJECTED DOSE PER LITRE

GROUP II

TIME IN HOURS	PLASMA		LYMPH		BILE	
	MEAN	RANGE	MEAN	RANGE	MEAN	RANGE
1/6	.02	.01 - .03	.05	0 - .13	-	—
1/3	.44	.10 - .72	.11	0 - .23	-	—
1/2	.54	.16 - 1.06	.46	.38 - .56	.38	.12 - .74
1	.68	.43 - 1.01	.42	.37 - .47	1.15	.49 - 2.05
1.5	.70	.51 - 1.04	.66	.44 - .80	1.39	.67 - 2.05
2	.83	.55 - 1.18	.89	.85 - .94	1.38	.73 - 2.08
2.5	1.00	.74 - 1.20	.82	.67 - 1.00	4.37	3.03 - 5.43
3	1.10	1.00 - 1.23	.97	.92 - 1.06	5.98	5.33 - 7.12
3.5	1.21	1.01 - 1.33	1.01	.85 - 1.11	8.74	5.61 - 11.05
4	1.47	1.10 - 1.95	1.02	.94 - 1.11	8.76	5.56 - 10.76
5	3.28	1.37 - 6.39	2.49	1.11 - 5.08	10.73	7.12 - 13.42
6	3.36	1.36 - 6.59	2.90	1.16 - 6.26	11.51	8.83 - 13.00
7	3.44	2.10 - 5.89	3.56	1.30 - 8.18	12.32	9.88 - 14.75
8	4.02	1.91 - 7.71	3.37	1.26 - 7.26	11.94	11.21 - 12.58
9	3.80	1.94 - 7.11	3.70	1.30 - 8.18	12.24	10.83 - 14.00
10	3.59	2.04 - 6.61	3.01	1.25 - 6.16	11.92	11.02 - 12.48
11	3.38	1.96 - 6.00	3.08	1.25 - 6.20	12.42	10.69 - 14.88
12	2.98	1.97 - 4.52	2.79	1.17 - 5.33	11.57	9.66 - 14.39

FIGURE VI

CONCENTRATION OF I^{131} IN HOURLY SAMPLES OF BILE, LYMPH, AND PLASMA IN 3 DOGS WITH CANNULATED COMMON DUCTS.

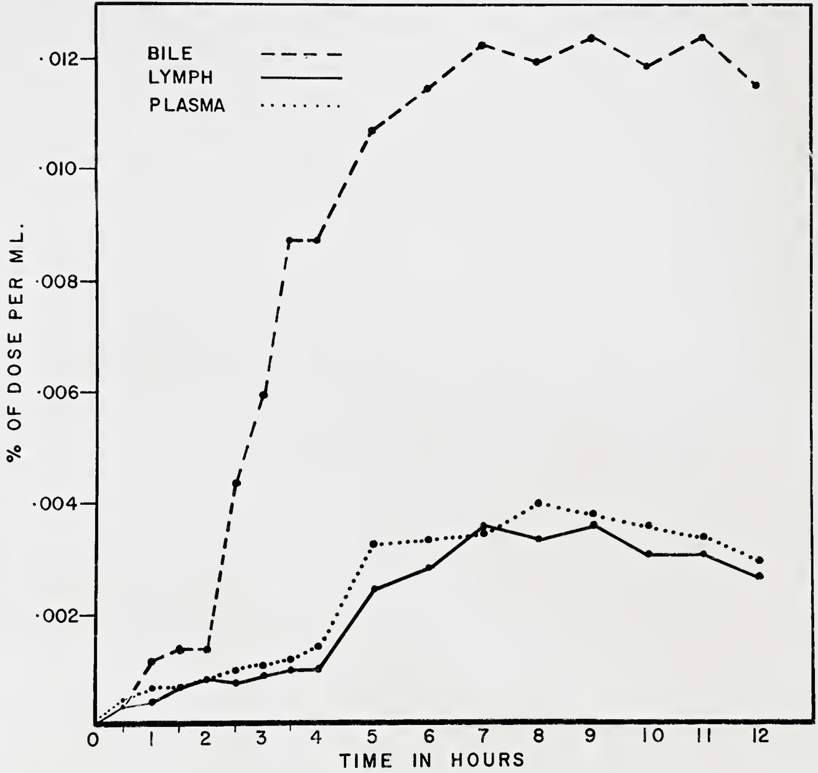


TABLE XI

MEAN CONCENTRATION OF RADIOACTIVITY IN PLASMA AND LYMPH IN 6 CONTROL DOGS, AND MEAN CONCENTRATION OF RADIOACTIVITY IN BILE IN 3 COMMON DUCT CANNULATED DOGS, EXPRESSED AS A PER CENT OF THE INJECTED DOSE PER LITRE

TIME IN HOURS	GROUP I 6 CONTROL DOGS		GROUP II 3 COMMON DUCT DOGS
	PLASMA	LYMPH	BILE
1/6	.80	.30	-
1/3	2.68	.80	-
1/2	4.03	1.68	.38
1	15.80	9.62	1.15
1.5	19.60	15.95	1.39
2	19.57	18.95	1.38
2.5	20.11	20.96	4.37
3	19.46	21.47	5.98
3.5	19.75	21.55	8.74
4	19.22	20.61	8.76
5	19.30	20.68	10.73
6	19.19	21.83	11.51
7	17.75	20.31	12.32
8	17.54	18.96	11.94
9	16.52	18.56	12.24
10	17.27	18.49	11.92
11	16.20	17.77	12.42
12	15.55	16.91	11.57

CHAPTER IV

DISCUSSION OF FINDINGS

The absorption of radioactive iodine (I^{131}), in peripheral blood plasma and in lymph from the thoracic duct was determined in 6 control dogs, and in 6 dogs with cannulated common bile ducts for a period of 12 hours.

No great difference in the concentrations of radioactivity in the plasma and in the lymph was observed in either group of dogs. A marked difference in the concentrations of radioactivity in the plasma and in the lymph was apparent between the two groups, with significantly higher values obtained from the control dogs in Group I. The highest concentrations in the control dogs in Group I occurred in the early period, between 2 to 6 hours, while the highest concentrations in the bile duct cannulated animals occurred later, between 8 to 12 hours (Tables III G, V).

Hourly and total volumes of lymph excreted during the 12 hour period were similar in the 2 groups of animals. However, radioactivity of the hourly and total lymph volumes was higher in the animals in Group I than in Group II dogs (Tables IID, IVC, VI).

Comparison of the concentrations of radioactivity of plasma among the control animals in groups I, III and IV, demonstrates that the effects of anesthesia and operative trauma did not markedly influence the absorption of radioactivity in these animals (Table VIII).

A markedly higher concentration of radioactivity was found

in the bile than in the lymph and plasma in 3 dogs in Group II with cannulated common bile ducts, in whom bile was collected for 12 hours. The concentration of radioactivity in the bile in these animals, was however, lower than the concentrations of radioactivity in the lymph and plasma in the control dogs in Group I (Tables XA, XI).

The role of the intestinal lymphatic circulation in the reabsorption of radioiodine via the 'afferent limb' of the enterohepatic circulation would appear, from this study, to be a passive one. The closely parallel concentrations of radioactivity in the peripheral plasma and lymph in animals in Groups I and II suggest that the lymph derived its radioactivity from a source common to the blood.

Ruegamer,[39] in 1953, reported a kinetic study of iodine metabolism in dogs under nembutal anesthesia. He concluded that a 4 hour period may be required for the attainment of equilibrium of radioactivity between plasma and other body fluids. During this period, outward diffusion into body fluids accounts for changes in I^{131} content in plasma and other tissues and fluids which are functions of the plasma concentration. Following this period (after equilibrium has been attained between plasma and other body fluids), the clearance of I^{131} into the thyroid gland, urine and other tissues proceeds at an exponential rate.

That radioactivity of lymph is a function of the radioactivity of the plasma is further supported by a recent (1956) report by Benson.[6] Water absorption from the intestine via portal and lymphatic pathways in rats was investigated utilizing tracer doses of D_2O. Benson observed that concentrations of the isotope in lymph and in arterial plasma were

identical at all times despite the changing concentrations of each simultaneously following administration of D_2O. Furthermore, the concentrations of isotope in lymph and in arterial plasma were the same, after a brief period of equilibration, whether the isotope was administered by stomach or by vein, either a peripheral or portal vein. Only a small proportion of the tracer substance was present in the intestinal lymph, and he concluded that the lymph derived its share of the isotope from a common blood source.

These findings and the results of the present study indicate that active reabsorption of radioactivity in intestinal lymph is unlikely. Nevertheless, this investigation demonstrates that radioactive material is present in lymph from the gastrointestinal tract, and is recirculated, through the thoracic duct, into the venous system. The magnitude of this 'passive' recirculation, however, in the 'afferent limb' of the enterohepatic circulation requires further investigation.

The effects of interference with 'afferent' and 'efferent limbs' of the enterohepatic circulation of thyroidal materials have been investigated previously, and are discussed in the introduction. In this study, only total radioactivity was determined, and the chemical state of the measured I^{131} was not investigated. As no exogenous thyroid hormones were introduced with the radioiodine, the results cannot be compared with those obtained by other observers of the enterohepatic circulation of radiothyroxine.[1,25,47] Even without measuring the endogenous hormones found with radioiodine, one may conclude from this study that a notable alteration in uptake of I^{131} may occur in the absence of bile.

An important factor affecting the uptake of I^{131} is stress. Badrick,[4] in 1954, demonstrated that a stress resulting in increased adrenocorticoid activity, significantly inhibited I^{131} uptake by the thyroid gland, partly due to the increased renal clearance of radioiodine. Telkka,[50] in 1954, demonstrated the histological depression of thyroid gland activity following ligation of the common bile duct in rats. He considered that the stress of the obstructive jaundice produced, was an important factor in his results.

In the light of this stress factor, an interpretation of experimental results of a radioiodine study must be carefully done. In the present investigation, nearly identical conditions (except for common bile duct cannulation) were produced in animals in Groups I and II, and it seems reasonable to assume that equivalent stress prevailed in both groups.

Markedly decreased concentrations of radioactivity in plasma and lymph during the 12 hour collection period, were observed in common bile duct animals in Group II, compared to the control animals in Group I. The curve of radioiodine uptake in plasma and lymph was altered in Group II animals as compared to the Group I dogs, with peak values delayed and prolonged in the former group (Table IIIG, Figure III).

These results were considered due to interference in the 'efferent limb' of the enterohepatic circulation produced by diversion of bile flow in animals in Group II.

A markedly higher concentration of radioactivity was found in the bile than in the lymph and plasma of three dogs in Group II in whom bile

was collected. This finding suggests an active secretion of radioactivity in the bile, confirming the earlier work[2] on enterohepatic circulation discussed in the introduction.

The uptake of radioactive iodine as a measure of thyroidal function is subject to many extrathyroidal influences. Such factors as impaired absorption, abnormal renal clearance, increased uptake by extrathyroid tissue, or an altered enterohepatic circulation may affect the utilization of a tracer dose. It would appear reasonable to assume that the enterohepatic circulation may play an important role in some clinical studies of thyroid function utilizing a tracer dose. Any clinical application from data derived from animal experimentation, however, must be undertaken with great caution.

The role of the enterohepatic circulation in iodine metabolism in man is now the subject of controversy. Information regarding the enterohepatic circulation in man is sparse, contradictory, and has been obtained from tracer studies performed in patients with liver disease or bile fistulae. The validity of interpretation from such cases may be questioned, but it has been the only available method of study.

In 1954, Mueller reported thyroidal I^{131} uptake greater than 50% in a series of euthyroid patients with severe liver disease. He concluded that if the enterohepatic circulation is significant in man, the net effect of liver disease on thyroid function may be unpredictable.

A study now in progress at the University of Alberta Hospital,[38] indicates a decreased uptake of radioactivity by the thyroid glands in patients with obstructive jaundice in whom tracer studies were done.

The paucity, however, of biliary excretion of radiothyroxine in the bile of patients with biliary fistulae in whom tracer studies were done[21] has led Berson[7] in a recent review (1956) to conclude that only a small fraction of thyroidal hormone participates in the enterohepatic circulation in man.

Thus, further investigations are required to elucidate the role of the enterohepatic circulation of thyroidal substances.

CHAPTER V

CONCLUSIONS

Absence of bile in the gastrointestinal tract of dogs significantly reduced the uptake of radioactive iodine (I^{131}) in plasma and lymph.

Interference in the enterohepatic circulation of radioiodine produced by cannulation of the common bile duct is believed responsible for this reduced uptake.

Nearly identical concentrations of radioactivity were found in the plasma and lymph in each of two groups of dogs.

A markedly higher concentration of radioactivity was found in the bile than in the plasma and lymph in three dogs with cannulated common bile ducts in whom bile was collected.

Anesthetic and operative procedures did not markedly affect the radioiodine uptake in the plasma.

These data suggest that clinical biliary obstruction of fistulae will produce an abnormal uptake of radioiodine given as a diagnostic tracer dose, and present further evidence of the importance of the enterohepatic circulation in the absorption of thyroidal substances.

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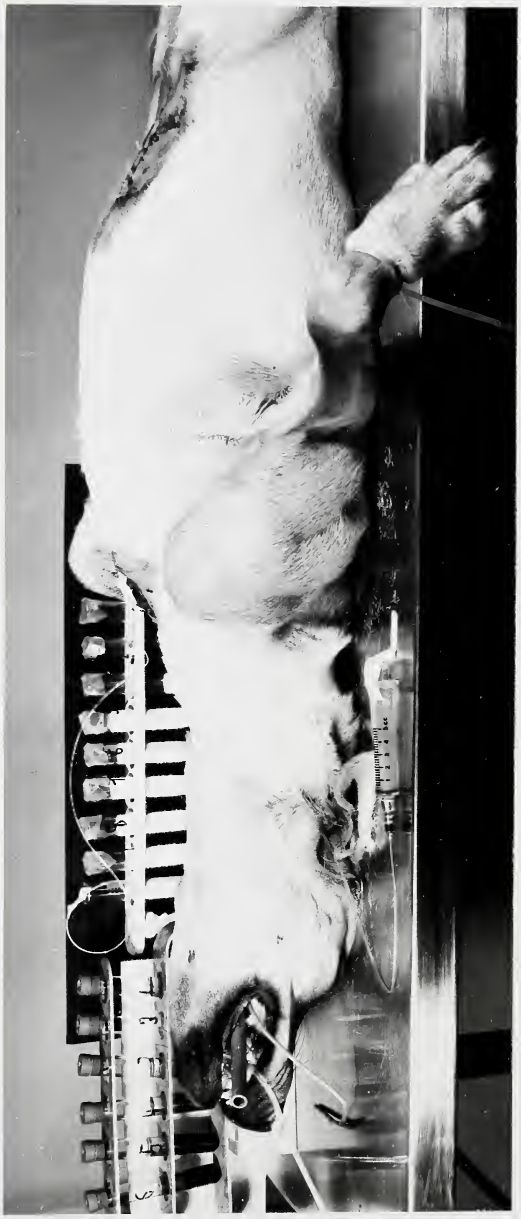
APPENDIX I

DIET			
COMPOSITION	PER CENT OF DIET	IODINE CONTENT IN MICROGRAMS %	IODINE CONTENT IN MICROGRAMS PER 100 GRAMS
Oats	20	5.2	1.04
Wheat	24	7.6	1.23
Beets	8	3.3	0.26
Wheat Germ	11	7.6	0.84
Soybean	11	6.3	0.69
Milk Powder	2.5	32.0	0.80
Meat Meal	15	30.0	4.50
Fish Meal	2	20.0	0.40
Cereal Grass	2	5.0	0.10
Potassium Iodide	0.5 oz. per ton		1400

The standard diet was Miracle Dog Food, supplied by Ogilvie Flour Mills, Edmonton, Alberta. Composition of this diet was known by analysis. Each dog received approximately a 100 Gram portion, of which 50 to 100 grams were consumed. The percentage of iodine in this table was obtained from a table of nutrition values,[3] and converted to micrograms in 100 Grams. The total iodine content in a 100 Gram portion was about 1410 micrograms. Consumption of an average 75 grams would then provide about 1000 micrograms of iodine per dog per day.

ILLUSTRATION OF COLLECTION OF SAMPLES OF PLASMA AND

LYMPH IN A CONTROL DOG (NO. 6), IN GROUP I



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